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# Needs for new plant-derived pharmaceuticals in the post-genome era: an industrial view in drug research and development

Ying Wang



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**Abstract** Plant metabolites have been the successful source of drugs and provided considerable value not only to the pharmaceutical industry but also to human health problems. Although pharmaceutical companies significantly decreased their activities in natural product discovery during the past few decades, various multidisciplinary approaches have been made to create new opportunities for finding innovative plant derived pharmaceuticals in post-genome era. Strategies to integrate the knowledge on medicinal plants into rational drug screening, the unique biodiversity of plant metabolites into random drug screening, and the chemical diversity of plant metabolites into combinatorial chemistry have been reviewed with concrete examples. Innovative biotechnologies in plant cell and tissue cultures, and the latest achievements in metabolic engineering and genetic modification should significantly improve the production sustainability and efficiency of plant-derived pharmaceuticals.

**Keywords** Biodiversity · Biotechnology · Chemical diversity · Metabolic engineering · Plant metabolites

## Introduction

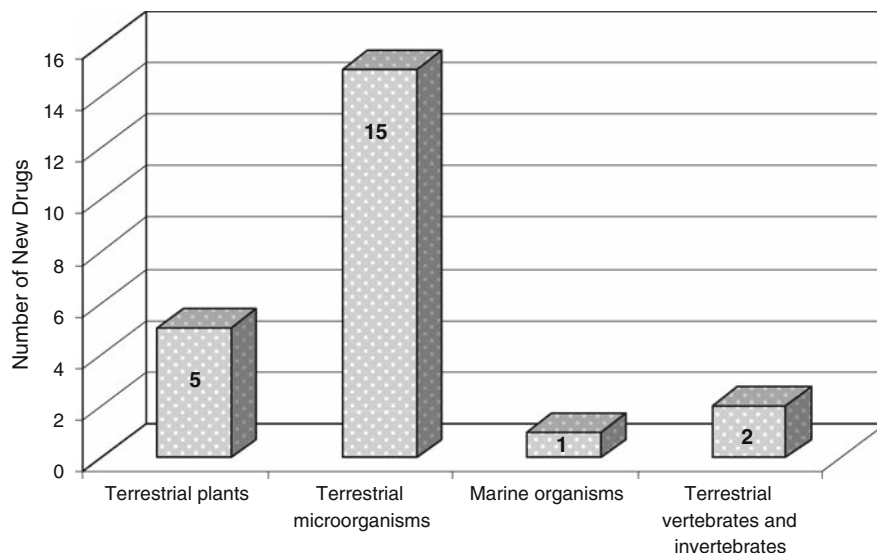
The natural world once served as the source of all medicinal agents, and plants provided most of these therapeutic entities. Documentation found in archeological excavations proves that various medicines extracted from plants were used as long ago as 2500 BC. Systematic study of these plants at the start of the 19th century has been one of the major factors in the development of drug research. Many important drugs, such as morphine, atropine, cocaine, codeine, papaverine, pilocarpine, digoxin, ergotamine, reserpine and artemisinin, were all discovered from plants in the last centuries.

Between 2000 and 2005, a total of 23 new drugs launched on the market originated from natural sources, including terrestrial plants, terrestrial microorganisms, marine organisms and terrestrial vertebrates and invertebrates (Chin et al. 2006) (Fig. 1). Five of these new drugs were discovered from plants or derived from plant metabolites (Fig. 2). They are apomorphine (**1**, a derivative of morphine from *Papaver somniferum*) for Parkinson's disease, tiotropium (**2**, a derivative of atropine from *Atropa belladonna*) for bronchospasm associated with chronic obstructive pulmonary disease, nitisinone (**3**, a derivative of leptospermone from *Callistemon citrinus*) used as a treatment of hereditary tyrosinaemia type 1 (HT-1), galanthamine (**4**, an alkaloid from *Galanthus nivalis*) as a selective acetylcholinesterase inhibitor for Alzheimer's disease treatment, and arteether (**5**, a derivative of artemisinin

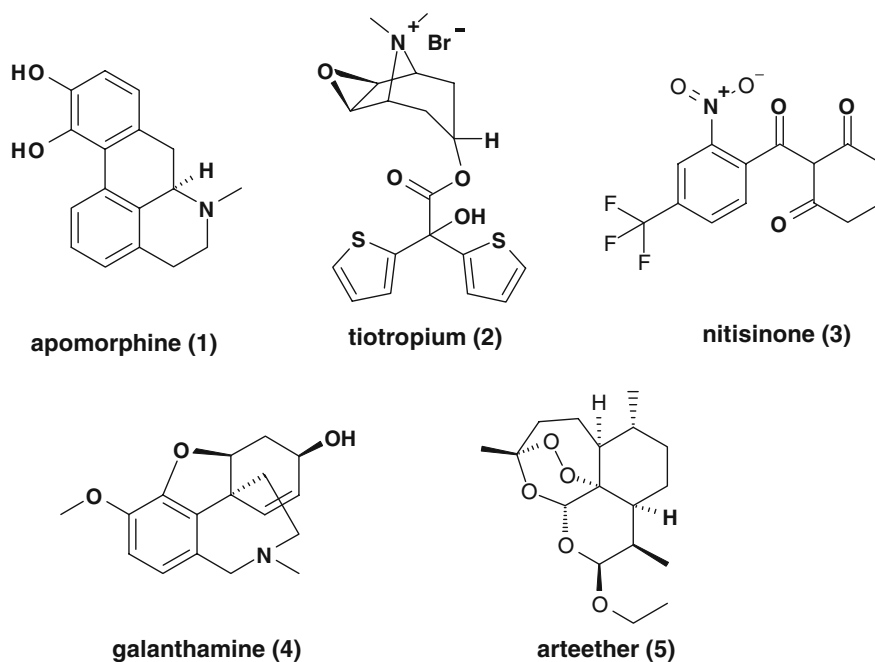
Y. Wang (✉)

Novartis Institute for Biomedical Research, Novartis Pharma AG, WSJ-507.6.04, 4002 Basel, Switzerland  
e-mail: ying.wang@novartis.com

**Fig. 1** Twenty-three new natural product drugs launched from 2000 to 2005



**Fig. 2** Five new drugs derived from plant metabolites (2000–2005)



from *Artemisia annua*) as an antimalarial agent. Together with other plant derived compounds in clinical trials (Chin et al. 2006), they demonstrated the importance of plant derived pharmaceuticals in modern drug discovery efforts.

It was estimated that the worldwide pharmaceutical R&D spending essentially tripled from roughly US\$ 10 billions to US\$ 30 billions in the years 1984–2003 (Koehn and Carter 2005). However, the pharmaceutical industry's productivity continues to be

dismal, and the New Chemical Entities (NCEs) hit a 24-year low in 2004 (Newman and Cragg 2007). The advent of genomics and the molecular biology revolution has permitted both the definition of new targets and the characterization of the genetic basis of disease states. The introduction of various powerful new technologies and innovative strategies have also greatly accelerated the pace of new drug discovery (Fishman and Porter 2005). All these progresses create immense opportunity for plant-derived

pharmaceuticals, which once served as the source of all medicinal agents in the ancient time and also provided many important therapeutic entities in contemporary drug research and development.

Although pharmaceutical companies significantly decreased their activities in natural product discovery during the past few decades, various multidisciplinary approaches have been made to create new opportunities for finding innovative plant derived pharmaceuticals in post-genome era. There can be no doubt that plants not only continue to retain their historical significance as important source of new drugs, but also are extremely useful as source of lead compounds for the design, synthesis and development of novel drug compounds in modern pharmaceutical research and development.

### **Integrate the knowledge with medicinal plants into rational drug screening**

Plant-derived herbal remedies have remained a vital part of traditional medicine for thousands of years. It has been estimated by the World Health Organization that approximately 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care. Plant products also play an important role in the health care systems of the remaining 20% of the population, mainly residing in developed countries (Cragg and Newman 2005a). The use of traditional medicine data can provide very valuable short cut by indicating plants with specific folk-medicinal uses which might be likely sources of biologically active compounds.

Recent investigations on medicinal plants used in traditional medicine have led to the discovery of many new drugs and hundreds of pharmacologically active substances for synthetic modifications. For example, *Artemisia annua* is an annual herb which has been used for many centuries in traditional Chinese medicine for the treatment of fever and malaria. Its effectiveness against strains resistant to chloroquine has attracted great attention. Artemisinin, a unique sesquiterpene with an endoperoxide moiety, has been successfully isolated and identified from this plant and is responsible for the therapeutic effects. As the most promising compound with outstanding antimalarial activity at low toxicity, artemisinin is an innovative cornerstone for anti-malaria therapy. In

Artemisinin-based Combination Therapies (ACT) recommended by the World Health Organization (WHO), artemether/lumefantrine is the only pre-qualified, fixed-dose ACT, which is a highly effective and well-tolerated antimalarial that achieves cure rates of up to 95%, even in areas of multi-drug resistance (Kuhn and Wang 2008).

Discovering novel targets not already hit by existing drugs is the prerequisite for the success to find innovative drugs. In target directed drug discovery, molecules acting on specific targets offer the advantage that the mechanism of drug action can be well understood and accurately monitored in preclinical research and clinical trials. Since the native use of medicinal plants in traditional medicine constitutes a kind of pre-existing clinical testing, investigations on their mode of action should increase the success probability to find novel targets. In another aspect, the specific folk-medicinal uses provide a very valuable short cut to biologically active compounds with particularly added advantages to related diseases.

Innovative technologies, such as high content screening, are emerging to assess the effect of a treatment on multiple pharmacological targets at cellular or organismic levels. As revisiting the ancient concept of botanical therapeutics, it is also worthwhile to notice that mixtures of interacting compounds produced by medicinal plants may provide important combination therapies that are simultaneously affect multiple pharmacological targets and provide clinical efficacy beyond the reach of single compound-based drugs (Schmidt et al. 2007; Qiu 2007; Ding 2003). If indigenous and traditional medical knowledge with plants can be integrated to the rational drug screening, it would be particularly advantageous for the multi-target identification and multi-component combination therapies.

### **Integrate the unique biodiversity of plant metabolites into random drug screening**

The angiosperms or flowering plants include more than 250,000 species of herbs, shrubs and trees. Together with the 700 species in gymnosperms, they constitute the higher plants (Spermatophytes) as the most diversified source of plant secondary metabolites. As chemical defenses against insect, microorganisms and other predators, higher plants are remarkable in

their ability to produce a vast array of diverse metabolites varying in chemical complexity and biological activity. Therefore, plant metabolites can effectively supplement chemical compound libraries and combinatorial synthetic efforts.

Despite the intensive investigation of terrestrial flora, it is estimated that only 5–15% of higher plants have been systematically investigated. The potential of large areas of tropical rainforests remains virtually untapped. With much of the biological diversity found in tropical and subtropical regions, the investigation of these resources requires multidisciplinary international collaboration in the drug discovery and development process (Cragg and Newman 2005b).

Chemotaxonomic approach is a potentially useful strategy in plant-derived drug discovery program. Chemotaxonomy relies upon the fact that taxonomically related plants often biosynthesize chemically similar secondary metabolites. Certain families are known to be rich in the kind of specific secondary compounds that tend to be medicinally relative. For example, biodynamically active alkaloids are particularly concentrated in some families (*Rubiaceae*, *Solanaceae*, *Leguminosae*, *Rauvolfiaceae*, *Berberidaceae*, *Papaveraceae* et al.) Thus, taxonomic correlations can provide useful clues in the drug discovery and screening of the numerous members of these taxonomic alliances might yield more positive results than random screening.

Crude plant extract libraries are usually generated by extracting plant material in two ways, either with a single solvent (or a mixture of solvents), or with multiple solvents, from non-polar to polar, in a sequential extraction (Buss and Butler 2004). Such classic plant extract libraries not only contain largest number of secondary metabolites, but also keep the smallest number of samples for screening. Although the past value of screening such plant extract libraries is indisputable, it is also closely associated with several major drawbacks, such as time-consuming follow up, unpredictable novelty of the secondary metabolites, interfering compounds for bioassays in the complex mixtures.

Solid phase extraction is a simple, rapid and relatively inexpensive technique to address the problem of extracts containing known, commonly occurring compounds. In the primary AIDS-antiviral screen at National Cancer Institute, 16,886 crude aqueous and organic solvent extracts from terrestrial plants were tested. Various solid phase extraction

cartridges, including Sephadex G-25, C<sub>4</sub> wide pore, C<sub>18</sub> narrow pore and polyamide resin, were used to remove anionic polysaccharides or tannins, and to determine the relative polarity of active constituents (Cardellina et al. 1993). The technique and strategy have been approved to be particularly useful for the dereplication and prioritization of HIV-inhibitory aqueous extracts.

With the rapid development of high throughput technologies in screening and combinatorial chemistry, some major global pharmaceutical companies diminished or even terminated their natural product research (Maureen Rouhi 2003a). For example, the natural product collections from GlaxoSmithKline, Lilly, Roche and Bayer have been transferred to Merlion in Singapore, Albany Molecular Research in the USA, Basilea Pharmaceutica in Switzerland and InterMed Discovery in Germany, respectively. By applying high throughput and automation technologies, pre-fractionated libraries have rapidly developed in the last several years, particularly in some small or middle sized biotechnology companies (Eldridge et al. 2002; Abel et al. 2002; Jia 2003).

At Sequoia Sciences Inc., the library production integrates automated flash chromatography, solid phase extraction, and high throughput parallel four channel preparative high performance liquid chromatography. The library containing 36,000 fractions was analyzed with eight channel liquid chromatography-evaporative light scattering detector-mass spectrometry (LC-ELSD-MS) at ultra-high throughput level (Eldridge et al. 2002). In another approach at BioLead GmbH (now a division of Galapagos), a semi-automatic process using the Isoleader System and ContiWeigher System allows the generation of about 8,000 fractions per month (Abel et al. 2002). In comparison with the crude extract libraries, there are several advantages with pre-fractionated ones, including easier follow up to isolate and identify active principles, better chance to find highly active minor metabolites, less false-positive due to the removal of interfering substances and higher quality with more novel compounds. The major problems are the high costs to generate and screen such libraries with dramatically increased size, and special miniaturized technologies needed in the process to isolate and identify active principles.

Another type of libraries consist of pure plant metabolites, which can be generated with high

throughput isolation and structure elucidation technologies (Maureen Rouhi 2003b). Instead of tracking biologically active compounds, this approach at AnalytiCon Discovery concentrates on retrieving from a biological material as many chemically interesting compounds as possible. High performance liquid chromatography was hyphenated with mass spectrometric, light scattering, and ultraviolet detection. High throughput structure elucidation is based on fragmentation patterns, molecular weights and database applications. One example in such approach was the collaboration project between AnalytiCon Discovery and former Aventis Pharma (now Sanofi-Aventis), in which a collection of 4,000 pure and non-redundant natural products were obtained within 18 months. A total of 400 compounds were randomly selected for structure elucidation, more than 39% of them not described in the Chapman & Hall Dictionary of Natural Products. In comparison with microbial samples, the creativity and productivity of plants were much higher: more than 30% of profiled extracts were selected for isolation and each plant materials yielded an average of 7.7 different compounds (Bindseil et al. 2001). In another approach at BioLead GmbH (now a division of Galapagos), a possibility was claimed to produce a library of more than 80,000 substances with an average purity of 85%. However, without giving any concrete data, such claim was neither validated nor realized (Abel et al. 2002).

For pure natural product library, there are also some professional providers, e.g. the Dutch SPECS

and BioSPECS and Interbioscreen in Moscow, who acquire pure natural products from scientists in academia and sell them profitably for screening. With such pure natural product libraries, the overall process from screening to validated hits is much faster than the time- and capacity-consuming bio-activity-guided isolation and purification procedures with the crude extract libraries or prefractionated libraries. It also enables active natural products to be evaluated on an equivalent basis to those from synthetic compound libraries. These advantages are particularly important for the integration of medicinal plant research into the contemporary drug discovery and development. However, the weaknesses of such approach are high investments to generate the libraries in early phase, limited access to broad biodiversity and little chance to find minor plant metabolites with interesting biological activities.

Detailed comparison of different plant metabolite libraries is summarized and presented in Table 1. Based on their advantages and disadvantages, an appropriate one or a combined approach can be well adapted to the drug discovery process.

### Integrate the chemical diversity of plant metabolites into combinatorial chemistry

The original goal of combinatorial chemistry was the synthesis of extremely large libraries through combination of available building blocks in a particular

**Table 1** Comparison of plant metabolite libraries in drug discovery

|               | Crude extract library                                                                                                                                                              | Solid phase extraction library                                                                                                                                                            | Fraction library                                                                                                                                                                                                                                         | Pure metabolite library                                                                                                                                                             |
|---------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Advantages    | <ul style="list-style-type: none"> <li>• Smaller library size</li> <li>• Larger biodiversity and chemical diversity</li> <li>• Easy to be prepared</li> <li>• Low costs</li> </ul> | <ul style="list-style-type: none"> <li>• Partially purified and enriched</li> <li>• Less interfering compounds for bioassays</li> <li>• Increased chance for minor metabolites</li> </ul> | <ul style="list-style-type: none"> <li>• Higher chance for minor metabolites</li> <li>• Easier to follow up</li> <li>• Better chance to find active minor metabolites</li> <li>• Much less “false positive”</li> <li>• More novel metabolites</li> </ul> | <ul style="list-style-type: none"> <li>• Fully compatible with HTS</li> <li>• Comparable with any pure compound library</li> <li>• Ensured metabolite novelty</li> </ul>            |
| Disadvantages | <ul style="list-style-type: none"> <li>• Interfering compounds for bioassays</li> <li>• Unpredictable novelty of metabolites</li> <li>• Time-consuming follow up</li> </ul>        | <ul style="list-style-type: none"> <li>• Unpredictable novelty of metabolites</li> <li>• Increased library size</li> <li>• Still time-consuming follow up</li> </ul>                      | <ul style="list-style-type: none"> <li>• Very large sample size</li> <li>• Increased screening costs</li> <li>• Limited biodiversity and chemical diversity</li> </ul>                                                                                   | <ul style="list-style-type: none"> <li>• Limited access to biodiversity and chemical diversity</li> <li>• High investment</li> <li>• Little chance for minor metabolites</li> </ul> |



synthetic scheme for high-throughput screening against the large number of new molecular targets. Following the maturation of combinatorial chemistry and compound library development for lead finding, it has been recognized that biological relevance and chemical diversity are more important than the library size (Piggott and Karuso 2004; Ortholand and Ganesan 2004; Koch and Waldmann 2005). Therefore, natural products have been considered as a hunting ground for combinatorial chemistry (Ganesan 2004). In comparison with viewing natural products as a stand alone approach distinct from combinatorial synthesis, it should be much more effective to adopt strategies that combine the strengths of both approaches. Three ways have been developed in current drug discovery, including utilizing natural product “privileged scaffolds” in combinatorial chemistry, assembling natural product like compounds through diversity oriented synthesis and creating new natural product derivatives by target oriented synthesis (Maureen Rouhi 2003c; Liao et al. 2003; Webb 2005).

Different from synthetics, there are a higher frequency of oxygen atoms and stereochemical elements like chiral centers and polycyclic, often bridged, carbon skeletons in natural products. In diversity oriented synthesis (DOS) inspired by natural products, the molecules in natural product-like libraries are skeletally diverse, structurally complex, stereochemically rich and densely functionalized. In the meantime, such libraries also offer the advantages of easy resynthesis and access to analogs (Shang and Tan 2005). Using these libraries to explore untapped regions of structure space will be certainly a valuable approach to identifying novel pharmacophores for challenging targets.

Galanthamine is an alkaloid from the bulbs and flowers of the *Caucasian snowdrop*, *Lycoris radiata*, *Galanthus woronowii* and related species. This plant metabolite was selected due to a range of functionality for diversity-generating reactions and a rigid polycyclic core that might lower the potential entropy penalty associated with protein binding. Its structure also allowed for the use of powerful biomimetic reactions in the solid-phase synthesis to prepare a 2527 membered library. The screening identified a molecule, named as secramine, from the library that perturbs the secretory pathway in mammalian cells (Pelish et al. 2001). Even though the library was

based on a plant metabolite scaffold which had no effect on the secretory pathway, diversity-oriented synthesis combined with phenotypic screening was successful in identifying an active molecule that may emerge as an important probe reagent for exploring protein trafficking.

Some exploratory approaches on plant metabolite-based combinatorial libraries are summarized in Table 2. Most of these combinatorial syntheses based on the plant metabolites invariably originated from the efforts by academic laboratories.

### **Integrate plant cell and tissue cultures into drug development and production**

Biotechnology is the application of scientific and engineering principles to the processing of materials by biological agents. Since 1970s, it has proved capable of generating enormous wealth and influencing every significant sector of the economy, and become a sustainable alternative for chemical industry (Gavrilescu and Chisti 2005). Biotechnology also offers an attractive opportunity to exploit the cell, tissue, organ or entire organism by growing them in vitro and to genetically manipulate them to get high value secondary metabolites.

The plant kingdom has historically been a major source of bioactive compounds for medicine, food additives, pigments, insecticides, cosmetics and fine chemicals. Unfortunately, their supply by the extraction from natural plants resources has some difficult issues: limited quantity of active metabolites in the plant, low plant growth rate, limited localization of active ingredients in the specific organs, and destruction of the natural resources. In comparison with the conventional cultivation, plant cell and tissue cultures are sustainable alternatives to the whole plant with clear advantages. They are independent of geographical and seasonal variations and environmental factors, ensure the continuous supply with uniform quality and yield, and provide efficient downstream recovery and product.

In comparison with microbial cells, larger plant cells size (20–50  $\mu\text{m}$ ) and their relatively inflexible cell wall make them sensitive to shear stress induced via impeller mixing in bioreactors. Their long growth circle results in significant longer doubling time (days) and therefore decreases fermentation throughput

**Table 2** Plant metabolite-based combinatorial libraries

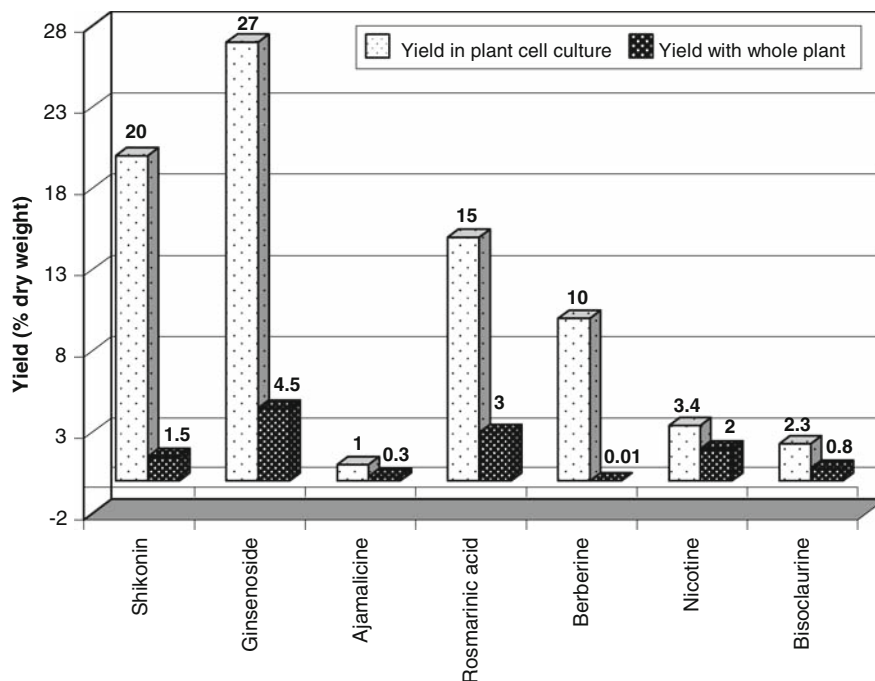
| Plant metabolite type | Scaffold or building block                                        | Combinatorial library                                                                                                                                                                                                                   | Reference                  |
|-----------------------|-------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------|
| Flavonoid             | Chalcones                                                         | A series of flavonoid derivatives obtained in high yields on the solid phase, demonstrating that these highly functionalized scaffolds can be used in the synthesis of natural product-based combinatorial libraries for drug discovery | Yao et al. 2007            |
| Alkaloid              | Ibogaine                                                          | A 75-member library of <i>N</i> ,6-disubstituted isoquinuclidines with a solution-phase method                                                                                                                                          | Levi et al. 2005           |
|                       | Huperzine A                                                       | Library produced both in the solution and on the solid polymer support                                                                                                                                                                  | Georgiev and Mincheva 2001 |
|                       | Galanthamine                                                      | Library prepared using biomimetic synthetic strategies                                                                                                                                                                                  | Pelish et al. 2001         |
|                       | Morphine                                                          | Synthesis of three combinatorial libraries accomplished to prepare 339 morphinan derivatives which were designed as selective opioid ligands                                                                                            | Ohno et al. 2002           |
| Diterpene             | Paclitaxel                                                        | Synthesis of the first 400-membered taxoid library in quantities of multimilligrams/member, using radiofrequency encoded combinatorial chemistry                                                                                        | Xiao et al. 1997           |
|                       | Labdane                                                           | 14-Deoxy andrographolide linked to 2-chlorotrityl chloride resin and followed by a series of solid-phase reactions                                                                                                                      | Biabani et al. 2001        |
| Triterpenoid          | Betulinic acid and Ursolic acid                                   | Triterpenoid-based scaffolds used for the generation of combinatorial libraries in parallel format using solid phase organic synthesis method for screening of antimalarial activity                                                    | Pathak et al. 2002         |
| Coumarin              | 7-Fluoro-4-methyl-6-nitro-2-oxo-2H-1-benzopyran-3-carboxylic acid | 7-Fluoro-4-methyl-6-nitro-2-oxo-2H-1-benzopyran-3-carboxylic acid as a novel scaffold for combinatorial synthesis of coumarins, derivatives synthesized in solid phase                                                                  | Song et al. 2004           |
| Lignan                | Aryltetralin                                                      | Parallel solution-phase synthesis of two libraries derived from a 1-aryltetralin                                                                                                                                                        | Dorbec et al. 2006         |
| Anthraquinone         | Anthraquinone-2-carboxylic acid                                   | Anthraquinone carboxylic acid as the building block for the acyl portion of a modified hexamer leads to significantly enhanced duplex stability in a combinatorial library of oligonucleotides                                          | Connors et al. 2003        |
| Saponin               | Trillin                                                           | A saponin library prepared by a random acetylation/random glycosylation sequence. The “double random” strategy should be useful for the facile preparation of other glycoconjugate libraries                                            | Yu et al. 2001             |

(weeks for one batch). Significant variability in product accumulation in plant cells creates special problems for downstream processing. In addition, cultured plant cells usually produce low amounts and altered profiles of secondary metabolites when compare with the intact

plant. Instability of the productive cell strains also gives difficulties to store and retrieve plant cell lines with increasing uncertainty (Terrier et al. 2007; Roberts 2007). All these well known drawbacks are the major challenges for the industrial applications of



**Fig. 3** Several plant metabolites accumulated in plant cell culture under optimized conditions even at higher level than in intact plant



plant cell and tissue cultures in a economic manner to produce plant-derived pharmaceuticals.

Successful strategies have been adopted to enhance the secondary metabolites in plant cell culture, including screening and selection of highly productive cell lines, addition of precursors, manipulation of nutrients to improve yield, optimizing the culture environment, and challenging by compounds of pathogenic origin as elicitors (Ramachandra Rao and Ravishankar 2002). With optimized conditions, several plant metabolites are accumulated in plant cell culture even at higher level than found in intact plant (Fig. 3), such as shikonin in *Lithospermum erythrorhizon*, ginsenosides in *Panax ginseng*, ajmalicine in *Catharanthus roseus*, berberine in *Thalictrum minor*, nicotine in *Nicotiana tabacum* and bisoclaurine in *Stephania cepharantha* (Dicosmo and Misawa 1995). A large number of secondary metabolites with important biological activities, i.e. vincristine, vinblastine, codeine, morphine, triptolide, triptolide, harringtonine, homoharringtonine, maytansine, camptothecin, artemisinin, ginkgolide B, hypericin, cephalomarine, hispidulin, bilobalide, crocin, coleol and paclitaxel, have been investigated or are even produced today in plant cell and tissue cultures (Zhou and Wu 2006).

If plant cell culture is not feasible for the production of some secondary metabolites derived from roots, another alternative with hairy root culture can be investigated. The major advantages of hairy root culture are genetic stability, fast growing ability and flexible incubation without needs for light and auxins. Many plant metabolites from medicinal plants with different structure skeletons have been successfully produced by hairy root culture, for example, anthraquinones from *Cassia* sp., triterpenes from *Glycyrrhiza uralensis*, diterpenes from *Salvia miltorrhiza*, flavonoids from *Glycyrrhiza glabra*, saponins from *Panax ginseng* and tropane alkaloids from *Duboisia leichhardtii*, polyacetylenes from *Bidens* sp. and volatile oil from *Artemisia absinthium* (Ramachandra Rao and Ravishankar 2002).

In the scale-up technology development of plant cell and tissue cultures, shikonin was the first secondary metabolite produced in commercial scale by Mitusi Petrochemical Industry Company in 1983. It also succeeded to produce berberine using plant cell culture technologies. In 1988, Nitto Denko Company successfully cultivated *Panax ginseng* cells in 20 m<sup>3</sup> bioreactors for the production of ginsenosides. Up to now, fourteen substances or products become produced commercially and semicommercially on the

basis plant cell cultures in bioreactors (Dicosmo and Misawa 1995; Frense 2007).

Production of paclitaxel (taxol), a complex diterpenoid from *Taxus brevifolia*, is a milestone accomplishment on the commercial scale by plant cell culture for pharmaceutical industry. Its worldwide sales were more than \$1.2 billion in 2003. Under a long-term collaborative relationship with Bristol-Myers Squibb, Phyton Biotech has successfully developed production of paclitaxel with world's largest dedicated plant cell culture production facility with large-scale fermentor capacity of up to 75,000 l. The process is under current Good Manufacturing Practice (cGMP) conditions. In 2004, The US Environmental Protection Agency awarded Bristol-Myers Squibb a Presidential Green Chemistry Challenge Award in the recognition of its development and the use of a more environmentally friendly way to manufacture, i.e. plant cell culture process, its anticancer drug taxol (Venkat 1998; Tabata 2006).

### **Integrate metabolic engineering and genetic modification into plant metabolite biosynthesis**

Due to highly complex structures, most of pharmaceutically important secondary metabolites from plants are mainly isolated from either wild or cultivated plants. To further increase chemical diversity for hit finding, biotechnological production of plant secondary metabolites can be enhanced by the treatment of undifferentiated cells with various elicitors. Advancement in bioconversion and combinatorial biosynthesis is promoting their application in lead optimization and drug development. Functional genomics approaches are accelerating plant gene discovery for biosynthetic pathways of plant metabolites and their regulation, which will also improve the production sustainability and efficiency of plant-derived pharmaceuticals.

With the rapid developments in molecular biological techniques and innovative strategies, combinatorial biosynthesis has been becoming a new tool in the generation of novel natural products, as well as for the production of biologically active natural products (Menzella and Reeves 2007). Recent achievements with the polyketide biosynthesis from microorganisms, especially in *Streptomyces*, have proved the potential of combinatorial biosynthesis (Hranueli et al. 2005). Since

different plants synthesize structurally similar but nevertheless diverse molecules, it can be expected that an enzyme with a certain substrate specificity isolated from one plant might encounter new but related substrates with introduced into another plant. By introducing genes involved in the biosynthesis of a given compounds isolated from one plant into another plant synthesizing related molecules, it can be hoped to produce new chemical structures not previously found in nature (Oksman-Cadentey and Inzé 2004; Oksman-Cadentey et al. 2004).

The major problem in combinatorial biosynthesis of plant metabolites is the gap of knowledge about genes and their regulation in plants of interest. Besides the crops with high economic values, only a few specific medicinal plant are currently under investigation, e.g. *Taxus baccata* and *Artemisia annua* (Julsing et al. 2006). However, the rapid advances in functional genomics and metabolomics should create unprecedented opportunities to use biochemical capacity of plants to design and to produce novel compounds.

Manipulation of biosynthetic pathways is also a powerful way to make natural product-like compounds. Although there was very limited precedence for precursor directed biosynthesis in plants, interesting findings have demonstrated on directed biosynthesis of alkaloid analogs in Madagascar periwinkle (*Catharanthus roseus*).

Vinblastine and vincristine are anticancer agents, and belong to approximately 130 terpene indole alkaloids (TIA) which were produced in a highly branched biosynthetic pathway from a common intermediate derived from tryptamine in the medicinal plant *C. roseus*. By feeding tryptamine analogues to both differentiated *C. roseus* and hairy root culture, the enzymes of this pathway can turnover unnatural substrates. This not only demonstrated that the TIA biosynthetic machinery can be used to produce novel alkaloid structures, but also highlighted the potential of this pathway for future metabolic engineering efforts (McCoy and O'Connor 2006).

Genetic engineering has become a particularly interesting method for manipulating metabolic productivity and unraveling regulatory aspects of plant metabolite biosynthesis (Verpoorte et al. 2007). Plant metabolic engineering could increase the level of valuable pathway intermediates or end products by overexpression of a rate-limiting enzyme or could

remove undesired metabolites through antisense, cosuppression or RNA interference (RNAi) reduction. In the genetical engineering of morphine biosynthesis in *Papaver somniferum*, overexpression of cyp80b3 cDNA resulted in an up to 450% increase of total alkaloids, whereas antisense-cyp80b3 cDNA expressed in opium poppy caused a reduction of total alkaloids in latex up to 84%. Therefore, cyp80b3 is a key regulation step in morphine biosynthesis and this provides practical means to genetically engineer valuable secondary metabolites in this important medicinal plant (Frick et al. 2007).

Recent efforts in genome sequencing give us the access to an incredible number of genes from various microorganisms and plants. The new biosynthetic functions discovered allow tapping into the synthetic potential of both microorganisms and plants. In the meantime, novel biosynthetic pathways and genes can be used for the synthesis of additional structures in engineered cells. The extraordinary complexity of plant biochemical capacity can also be explored with plant metabolomics and system biology. Particularly, the key genes may be identified by state-of-the art genomics tools, in combination with metabolic profiling, for the production of plant metabolites (Verpoorte and Memelink 2002; Watts et al. 2005; Oksman-Caldentey and Saito 2005).

Production of the antimalarial drug precursor artemisinic acid in engineered yeast is a promising approach in genetic engineering (Ro et al. 2006). The engineered yeast (*Saccharomyces cerevisiae*) is capable of producing high titres of artemisinic acid up to 100 mg l<sup>-1</sup>. However, further optimization and technology development are still needed to raise artemisinic acid yield to a higher level and to scale-up for industrial production, which could eventually meet significantly increased demand for artemisinin (Kuhn and Wang 2008). In comparison with the current process with wild or cultivated plants, this could be a very attractive production alternatives to ensure the supply of artemisinin in the future.

## Conclusion

Natural products have been a successful source of drugs and provided considerable value not only to the pharmaceutical industry but also to human health problems. By integration in most technologies and

concepts of modern drug discovery, many new drugs or drug candidates still originated from natural products or derivatives thereof, illustrating their potential for innovative treatments of human diseases (Petersen and Amstutz 2008). Although natural product research has experienced a steady global decline, plant metabolites remain an important source for new pharmaceuticals with its unique biological and chemical diversity. To meet the challenges in drug discovery and development, multidisciplinary approaches have been effectively creating new opportunities to find innovative plant derived pharmaceuticals in post-genome era, including the integration of plant natural product research with high throughput screening, rationale drug screening, combinatorial synthesis, plant cell culture technology, and metabolic and genetic engineering.

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